METABOLISM OF RIBOSOMAL RNA IN MUTANTS OF *ESCHERICHIA COLI* DOUBLY DEFECTIVE IN RIBONUCLEASE III AND THE TRANSCRIPTION TERMINATION FACTOR RHO

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ABSTRACT

To determine if proteins RNase III and rho, both of which can determine the 3′ ends of RNA molecules, can complement each other, double mutants defective in these two factors were constructed. In all cases (four *rho* mutations tested) the double mutants were viable at lower temperatures, but were unable to grow at higher temperatures at which both of the parental strains grew. Genetic analyses suggested that the combination of the *rnc rho* (RNase III⁻ Rho⁻) mutations was necessary and probably sufficient to confer temperature sensitivity on carrier strains. Physiological studies showed that synthesis and maturation of rRNA, which is greatly affected by RNase III, as well as other RNAs, was indistinguishable in *rnc rho* strains as compared to *rnc rho*+ strains, thus suggesting that RNase III and rho do not complement one another in determining the 3′ ends of RNA molecules. In *rnc rho* strains, however, the newly synthesized rRNA failed to accumulate. Thus, decay of rRNA could be the reason for the temperature sensitivity of the double mutant strains. These experiments suggest that RNase III and rho can both protect rRNA from degradation by cellular ribonucleases. They also point to the possibility that the nucleotide sequences involved in the determination of the 3′ ends of RNA molecules by these two factors are not identical.

A number of studies have shown that the rho factor, which is determined by the SuA gene (RICHARDSON, GRIMLEY and LOWERY 1975), can affect termination of transcription by RNA polymerase *in vitro* (ROBERTS 1969; DE CROMBRUGGHE *et al.* 1973) and *in vivo* (DAS, COURT and ADHYA 1976; KORN and YANOFSKY 1976a,b; the suA gene is now called *rho.*) Studies with ribonuclease III *in vitro* (DUNN and STUDIER 1973a; GINSBURG and STEITZ 1975) and *in vivo* studies with mutants defective in RNase III (DUNN and STUDIER 1973a; APIRION, NEIL and WATSON 1976b; GEGENHEIMER, WATSON and APIRION 1977) show that this enzyme can determine the 3′ ends of T7 early messengers, as well as of rRNA molecules of *E. coli.* Moreover, studies by LOZERON, ANEVSKI and APIRION (1977) showed that RNase III and rho could affect a bacteriophage λ early transcript near the 3′ end of the N protein message. In the presence of rho, the transcript is terminated somewhere beyond the N protein message, while in

the absence of rho this transcript can be elongated much further, but RNase III cuts it in the vicinity of the rho-mediated termination event.

These observations opened up the possibility that RNase III and rho could both affect the termini of similar molecules. To test the possibility that RNase III and rho could substitute for one another in the determination of 3′ ends of rRNA molecules, *rnc rho* double mutants were constructed. The construction of such strains and some of their properties are described here. The results suggest that these two proteins do not substitute for one another in the determination of termini of rRNA molecules, but that both might interact indirectly by affecting the stability of RNA molecules.

MATERIALS AND METHODS

All of the strains that we used are described in Table 1 and Figure 1. All of the techniques we employed have been previously described. Crosses, prolonged matings and transductions were carried out according to LENNETTE and APRION (1971). Transfer of F′ to F⁻ cells, when no selection against the F⁻ cells was possible, was carried out as suggested by MILLER (1972). Strains were assayed for RNase III either by the poly (I) · poly (C) assay (APIRION and WATSON 1974), or by the analysis of short term (20 to 30 min) $^{32}P_i$ labeled RNA on 3% polyacrylamide gels (APIRION, NEIL and WATSON 1976b). (In the last reference, a full correlation was established between the appearance of the large rRNA molecules, 25S and 30S, and the RNase III⁻ phenotype.) Gel electrophoresis was carried out as described by GEGENHEIMER, WATSON and APIRION (1977). Strains tested for *rho* contained a *lac* polar mutation, the polarity of which was suppressible by a *rho* mutation. These strains were replicated to a minimal medium containing 0.1% melibiose as the sole carbon source, and they were incubated at 37° for at least three days (KORN and YANOFSKY 1976a).

All media used were previously described (APIRION 1966; APIRION and WATSON 1975; LENNETTE and APIRION 1971). Growth of cells and their preparation for analysis in sucrose gradients, and the sucrose gradients were according to RON, KOHLER and DAVIS (1966).

RESULTS

Construction of rnc rho *(RNase III⁻, Rho⁻) double mutants*

In order to construct *rnc rho* strains, strain N3703 (F′ *rnc rho*⁺) was crossed to strain N2112 (*rnc*⁺ *rho*) in a prolonged mating at 37°. The selection was for L-valine resistance (against strain N3703) and for *Trp*⁺ (against strain N2112) in a minimal medium supplemented with nicotinic acid and 200 μg/ml of L-valine. The cross was plated at 37°. Fifty of the recombinants were analyzed, of which only two were Nic⁻. Since the *nadB* (Nic⁻) marker is co-transduced with *rnc* (APIRION and WATSON 1975; STUDIER 1975), recombinants that received the *nadB* marker from the *rnc* donor strain are also likely to be *rnc*. The two Nic⁻ recombinants were also Ts⁻ at 43°, unlike either of the parental strains (tested by replication). Both these Nic⁻ Ts⁻ recombinants kept the *rho* allele as indicated by the fact that, while they were still Lac⁻, they could utilize melibiose as the sole carbon source. (The point of origin of the F′ carried in strain N3703 is far enough from the *lac* gene to ensure that in a cross where the selective markers do not cover the *lac* gene it will be contributed mainly by the F⁻ parental

TABLE 1

Strains used

Strains	Markers	Source
D10	*metB1 rna-10*	R. GESTELAND
BE280	*ilv-280 trp-37 tna-4 phoS3 lacZ13 xyl-13 rpsL106*	OTSUJI*
#2054	*leu trpE9851 rho-100*	D. MORSE
N2076	*thi-1 rpsL9 argH1 nadB4 lacY1 gal-6 malA1* λ^R *xyl-7 ara-13 mtl-2*	APIRION and WATSON (1975)
N2093	as N2076, but *rnc-105 mal*+	
N2095	as N2093, but *rnc*+	
N2111	W3110 *trpR lacZu118* val^r *azi trpA9761 trpE9829* F+, F′ or Hfr	KORN and YANOFSKY (1976a)
N2112	as N2111 but *rho-101*†	KORN and YANOFSKY (1976a)
N2113	as N2111 but *rho-102*	KORN and YANOFSKY (1976a)
N2114	as N2111 but *rho-103*	KORN and YANOFSKY (1976a)
N2115	as N2111 but $R17^R$	
N2116	chromosome like in N2115 and contains F152	F152/KL253 × N2115
N2324	*lacZu118 rho-101 ilv*(Val-R) *nadB4 rnc-105*	N3703 × N2112
N2326	*rnc-105 thi-1 nadB4 ilv-280 rpsL106*	
N2328	*lacZu118 thi-1 nadB ilv-280 rpsL106 rnc-105*	N2116 × N2326
N2335	*thi*⁻ *nadB4 lacZu118 rnc-105 rpsL106 rho-101*	N2112→N2328‡
N2336	as N2335 but *rho-102*	N2113→N2328
N2337	as N2335 but *rho-103*	N2114→N2328
N3703	chromosome as in N2093 and contains F15	F15/KL110 × 2093
F15/KL110	*argG6 metB1 his-1 leu-6 thyA23 recA1 rpsL104*	episome F15 K. B. Low*
F152/KL253	*tyrA2 thi-1 pyrD34 his-68 trp-45 recA1 rpsL118*	episome F152 K. B. Low*

* Strains supplied by B. J. BACHMANN, Yale University, Coli Genetic Stock Center.
† The *rho* alleles were designated *101*, *102* and *103*, as suggested by KORN and YANOFSKY (1976b).
‡ Denotes a transduction; arrow leads from donor to recipient.

strain. We found that the use of the melibiose test to discriminate *lac* (polar) *rho* from *lac* (polar) *rho*+ is useful only at 37°, since at 30° both *rho*+ *lac* and *rho lac* strains can utilize melibiose.) In order to examine the segregation of the *rnc* marker, 24 colonies from the cross were tested for RNase III by enzymatic assays and on gels after labeling cells with ^{32}Pi. The 22 Ts+ strains were RNase III+, while the two Nic+ Ts⁻ strains were RNase III⁻. This experiment, therefore, suggests that *rnc rho* double mutants are viable, but that this combination is deleterious to the cell at higher temperatures.

To ascertain whether these two mutations are necessary and sufficient to cause the temperature sensitivity, a number of experiments were carried out. First, an *rnc-105 ilv lac* (polar) strain was constructed. (Since *rho* is co-transduced with *ilv*, *rho* alleles can be transferred by a P1-mediated transduction into such a

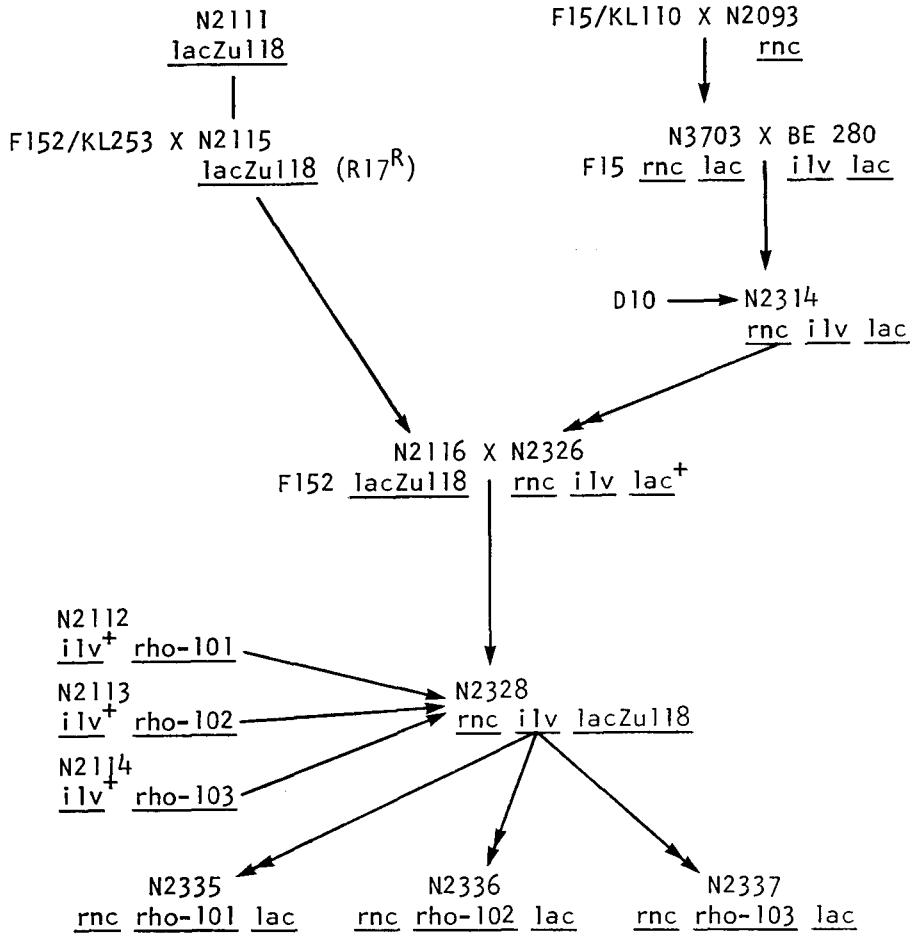

FIGURE 1.—Geneology of some of the strains studied. A straight line designates derivation by a spontaneous mutation. A × designates a mating, and an arrow leads to the recombinant. When recombinants were derived by transduction, a single arrowhead leads from the donor to the recipient and a double arrowhead from the recipient to the transductant. The relevant genotype is designated below each strain. For further details about genotypes see Table 1.

strain, and the resultant Ilv+ transductants can be tested with melibiose to find out whether or not they received the *rho* mutation). The strain N2328 was constructed in the following way (see Figure 1). First, *rnc-105* was transferred to an *ilv* strain (N3703 × BE280), and thus an *ilv rnc* strain was constructed. Since this strain was *lac*, a *lac*+ allele was transferred to it from a Lac+ strain (D10) by a P1-mediated transduction. This new strain, which is *lac*+ *rnc ilv*, was designated N2326. In parallel, strain N2111 was cured of its maleness by selecting a strain from it (N2115) that is resistant to the male-specific RNA phage R17. An F′ plasmid, which could mobilize the *lac* gene, was transferred to this strain. The new strain N2116 was crossed to N2326 by a prolonged mating and *lac rnc nadB*

ilv recombinants were isolated. One of these recombinant strains was designated N2328. The geneology of some of the strains used in these studies is summarized in Figure 1.

In a series of P1-mediated transductions, strain N2328 *ilv lacZu118 rnc-105* was the recipient, and four different *rho* mutants were the donors. In each case selection was for *Ilv*$^+$ transductants (selection was carried out at 30° and 37°), and at least 30 transductants from each temperature were purified and tested. The four *rho* mutants included the three *rho* alleles *101*, *102*, and *103* described by KORN and YANOFSKY (1976a), as well as the *rho* allele included in the SuA mutant isolated by MORSE and GUERTIN (1972). The *rho* allele in this strain will be referred to as *rho-100*, while the *rho* alleles isolated by KORN and YANOFSKY (1976a) are referred to by the numbers suggested by these authors (*rho-101*, *102* and *103*; see Table 1 and KORN and YANOFSKY 1976b).

The results of these experiments are summarized in Table 2. As no significant differences were observed between transductants isolated at 30° or 37°, the results (presented in Table 2) are those from both temperatures. (The colonies included in Table 2 were purified twice before being tested.) In Table 2, the phenotype of the transductants is described only with respect to their ability to grow at 43° and to utilize melibiose. (All the transductants were tested to assure that they still carry all the unselected markers of the recipient strain and that all remained RNase III$^-$.) Only two out of four possible classes of *Ilv*$^+$ transductants appear. The majority class remained Mel$^-$ Ts$^+$, like the recipient strain N2328, while the minority class was Mel$^+$ Ts$^-$. Thus, the *rnc rho* recombinants, which include the *rho-101*, *rho-102* and *rho-103* alleles, fail to grow at 43°, while the *rnc rho-100* recombinants were partially affected in their ability to grow at 43°.

Thus, this experiment demonstrated that every transductant that received the *rho* mutation became Ts$^-$, and the earlier result from the cross N3703 × N2112 was corroborated. Together with the results of the first cross, this suggests that both mutations *rnc-15* and *rho* are necessary for the observed interaction.

To determine if both mutations are sufficient to cause this interaction, the *rnc* mutation in the double *rnc rho* strains was replaced by an *rnc*$^+$ allele. This was

TABLE 2

Interaction between rnc *and* rho *mutations*

Donor	No. of Ilv$^+$ transductants analysed	Mel*+ Ts$^+$	Mel$^-$ Ts$^-$	Mel$^+$ Ts$^-$	Mel$^-$ Ts$^+$
rnc+ *rho-101* (N2112)	139	0	0	17	122
rnc+ *rho-102* (N2113)	163	0	0	8	155
rnc+ *rho-103* (N2114)	120	0	0	15	105
rnc+ *rho-100* (#2054)	113	0	0	14†	99

* Mel+, Mel−; able or unable to utilize melibiose.

† These transductants are much less temperature sensitive than all the others.

In all the experiments the recipient was strain N2328 (*rnc-105 rho*+). Selection was carried out for Ilv+. It was achieved by plating the P1 infected recipient cells on minimal medium containing thiamine and nicotinic acid.

accomplished either by transfer of an *rnc*+ gene from a strain containing an appropriate F′ plasmid or by transducing into these strains an *nadB*+ allele and testing the *Nic*+ transductants for RNase III and temperature sensitivity. The plasmid transfer is meaningful, since the *rnc* mutation is recessive (APIRION, NEIL and WATSON 1976b). The three combinations *rnc-105 rho-101*, *rnc-105 rho-102* and *rnc-105 rho-103*, were analyzed in this way. In all cases the RNase III+ transductants were Ts+, while the RNase III- transductants were Ts-. When the *rnc*+ episome was transferred to *rnc rho* strains, they also became Ts+. Thus, these experiments indicate that these two mutations, *rnc* and *rho*, are necessary and sufficient for this interaction. Since these two mutations, *rnc* and *rho*, are far apart on the chromosome of *E. coli* (BACHMANN, LOW and TAYLOR 1976), 55 min and 83 min respectively, in no case could removal of one mutation affect the other.

Some characteristics of the rnc rho *double mutants*

The double *rnc rho* strains are temperature sensitive at 43° in broth medium or minimal medium. This can be seen either by replication, by colony formation or by following the growth of the strains when they are incubated at 30° or 37° and transferred to 43°.

In Table 3, the colony-forming ability of some of the doubly mutant strains and the parental strains were tested. It is quite clear from these results that the double mutants fail to form colonies at 43°, while each of the parental strains do so. In Figure 2, the growth at 30° and 43° of one *rnc rho* mutant and its parental strains is shown. It is clear that the double mutant when transferred to 43° grows (or increases its mass) for only about two hrs and ceases growing well before stationary phase, while the parental strains keep growing exponentially till they reach the stationary phase. All three double mutant strains behaved similarly (*rnc rho-101*, *rnc rho-102*, *rnc rho-103*). (In the medium used in Figure 2, growth is exponential only to an A_{560} of about one. Therefore the faster growing strains reached this value earlier, and the values observed for these strains at later times are not shown.)

TABLE 3

Colony formng ability

Strain	Temperature of incubation 43°	44°
rnc+ *rho*+ (N2111)	1.15	0.82
rnc+ *rho-101* (N2112)	0.93	1.19
rnc+ *rho*+ (N2095)	0.91	0.98
rnc rho+ (N3703)	1.21	0.32
rnc-105 rho-101 (N2324)	0.03	< 0.001
rnc-105 rho-101 (N2335)	0.02	< 0.001

Cultures were grown in rich medium to about mid-log phase, diluted, and plated on rich medium plates, and incubated at 37°, 43° and 44°. Colonies were counted after three days. The number of colonies appearing at 37° was considered as one. The cultures contained about 2×10^8 colony-forming units per ml when viability was tested at 37°.

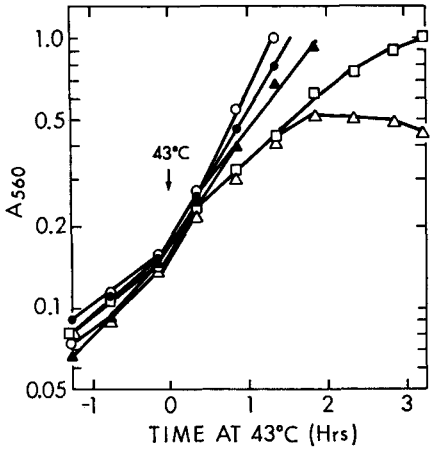

FIGURE 2.—Growth of strains. Cultures from a number of strains were grown at 30° in tris minimal medium containing 0.2% glucose and 0.6% (w/v) peptone, plus all the requirements of these strains. The absorbancy was determined with a PQ2 Zeiss spectrophotometer. Cultures were shifted at about the same density to 43°. Symbols used, ○—○, N2111, *rnc+ rho+*; ●—●, N2076, *rnc+ rho+*; ▲—▲, N2112, *rnc+ rho-101*; □—□, N3703, *rnc rho+*; △—△, N2324, *rnc rho-101*.

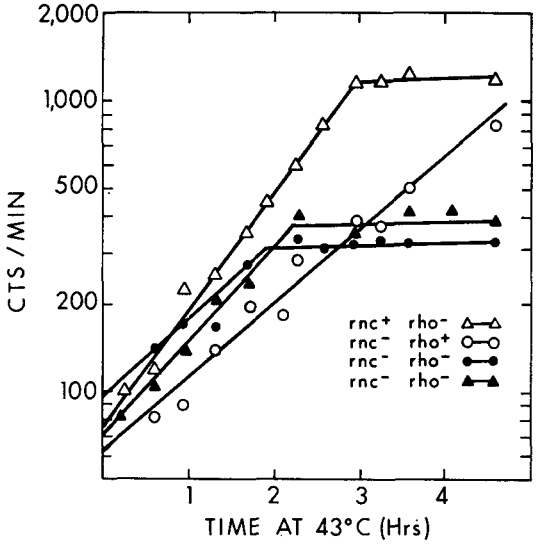

FIGURE 3.—Accumulation of protein in various strains. Strains were grown at 30° in the same medium as in Figure 2. 0.5 μC/ml of [^{3}H]-phenylalanine (350 mC/mmole) and 12 μg/ml of nonradioactive phenylalanine were added to the cultures. 0.5 ml samples were withdrawn from the cultures at the indicated time intervals. The cultures were transferred to 43° when their absorbance reached to an A_{560} of about 0.1. The withdrawn samples were added to equal volumes of 10% trichloroacetic acid. The samples were heated at 90° for 20 min and filtered and washed through glass fiber filters. The filters were washed with 0.1 N HCl, dried and counted using a toluene-based scintillation fluid. Strains used: N2112 (*rnc+ rho*), N3703 (*rnc rho+*), N2324 (*rnc rho*) ●—●, and N2336 (*rnc rho*) ▲—▲. Strain N2112 reached its stationary growth phase when the accumulation of protein leveled off.

In measurements of RNA and protein synthesis, we found that while protein synthesis continues for about 120 to 150 min after the shift (Figure 3); RNA accumulation stops somewhat earlier (Figure 4). In Figure 4, RNA synthesis was monitored by measuring the incorporation of [^{3}H]-uracil into cold trichloracetic acid precipitable material. Since some of this material could represent DNA, we repeated such experiments after treating the samples with 0.5 N NaOH at 37° for two hrs to degrade the RNA. Such experiments showed that the amount of uracil incorporated into DNA was negligible (less than 2%) regardless of the temperature at which the experiments were carried out, thus indicating that the results shown in Figure 4 pertain to RNA.

In Figure 4 it can be seen that strains N2324 and N2335 (both are *rnc-105*, *rho-101*) when shifted to 43° lose net RNA, and therefore clearly degrade their rRNA (mRNA usually does not accumulate). The other two doubly mutant

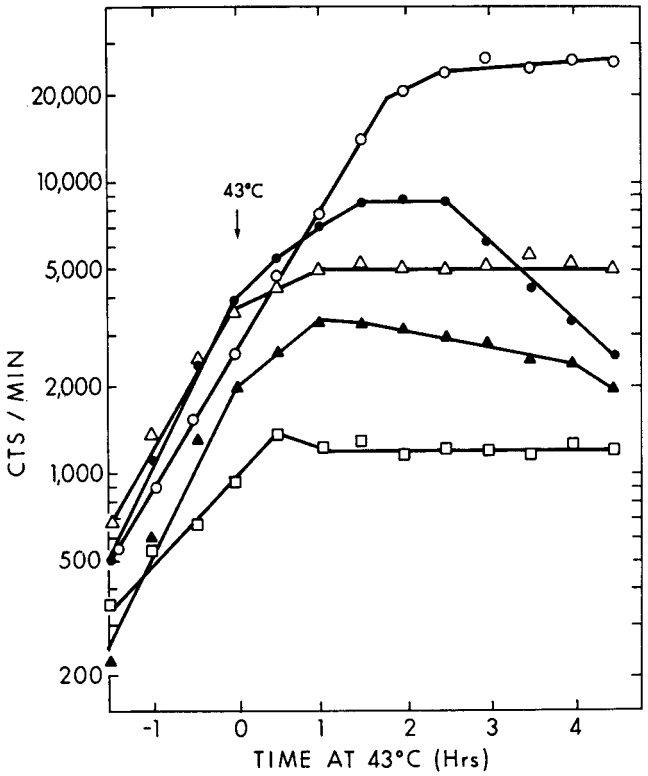

FIGURE 4.—Accumulation of RNA in different strains. Cells were grown in the same medium and temperature as described in the legend to Figure 2. When the cultures reached absorbance of about 0.05, ^{3}H-uracil (1 μC/ml, 300 μC/mmole) and 5 μg/ml of unlabeled uracil were added to the cultures. At the indicated time intervals, 0.3 ml samples were withdrawn into equal volumes of 10% trichloroacetic acid. The samples were filtered and processed as described in the legend to Figure 3. ○—○, N2112, *rnc+ rho-101*; ●—●, N2324, *rnc-105 rho-101*; ▲—▲, N2335, *rnc-105 rho-101*; △—△, N2336, *rnc-105 rho-102*; □—□, N2337, *rnc-105 rho-103*.

strains (N2336 and N2337) stop accumulating RNA at 43°. They also degrade their RNA, since it is being synthesized at 43° (see below).

Synthesis of rRNA in rnc rho *strains*

Since *rnc rho* strains grow at 37°, obviously all the essential RNA molecules must be matured normally in such strains. Indeed, numerous scanning of RNA molecules separated in polyacrylamide gels failed to reveal any differences in the pattern of RNA synthesized in *rnc rho* strains as compared to *rnc rho*$^+$ strains. Small RNAs were analyzed on tandem 5–12% gels (APIRION, NEIL and WATSON 1976b) and large RNAs on 3% gels (GEGENHEIMER, WATSON and APIRION 1977). (The pattern of newly synthesized RNA in *rnc* strains is different from that seen in *rnc*$^+$ strains, GEGENHEIMER, WATSON and APIRION 1977.)

Since *rnc rho* strains fail to grow at elevated temperatures, the possibility existed that at these temperatures the synthesis of rRNA is abnormal. The experiments described in Figures 3 and 4 suggest that even though RNA stops accumulating at the nonpermissive temperature in *rnc rho* strains, protein synthesis does not stop and, therefore, by inference, RNA synthesis does not cease. The possibilities are that either the synthesis of stable RNA is differentially blocked or that it is synthesized and degraded. The experiments described below suggest that the latter is correct.

In a series of experiments, all the parental strains, as well as all the doubly-mutant strains, were grown at 37°, shifted to 43°, and at various time intervals the RNA was labeled with ^{32}Pi. Cells were lysed in a sodium dodecyl sulfate-containing buffer (GEGENHEIMER and APIRION 1975), and their contents were analyzed by electrophoresis in slab gels. The gels were dried, autoradiographed and photographed. In these experiments cells were labeled even as late as 150 to 180 min after the shift to 43°. In all these experiments, the results were invariably similar—rRNA was synthesized and was properly processed to 16S rRNA. (Similar results were obtained when cells were grown at 30° prior to the shift.) Our method of handling the cells and running the gels allows no clear distinction to be made between the p23 (precursor 23S rRNA) and m23 (mature 23S rRNA; GEGENHEIMER and APIRION 1975; GEGENHEIMER, WATSON and APIRION 1977). Some of these experiments are presented in Figures 5 and 6. (The patterns of rRNA synthesis observed in *rnc*$^+$ *rho*$^+$ strains are identical with those observed in *rnc*$^+$ *rho* strains, and therefore in Figures 5 and 6 the patterns of rRNA observed in *rnc*$^+$ *rho*$^+$ strains are not shown.) The RNA patterns of small and large RNA molecules synthesized at various times before and after the shift to the nonpermissive temperature were compared and in no case were significant differences observed between the RNA synthesized in the double mutant strains and in the parental *rnc rho*$^+$ strain. Thus, it is clear that while rRNA is properly synthesized and processed, it is not accumulated (see Figure 4).

To ascertain if newly synthesized RNA, and specifically rRNA, is being differentially lost in *rnc rho* strains at the nonpermissive temperatures, strains were pulse labeled with [^{3}H]-uracil for 90 sec, and RNA and DNA synthesis were blocked with rifampicin (400 μg/ml) and nalidixic acid (30 μg/ml) respectively,

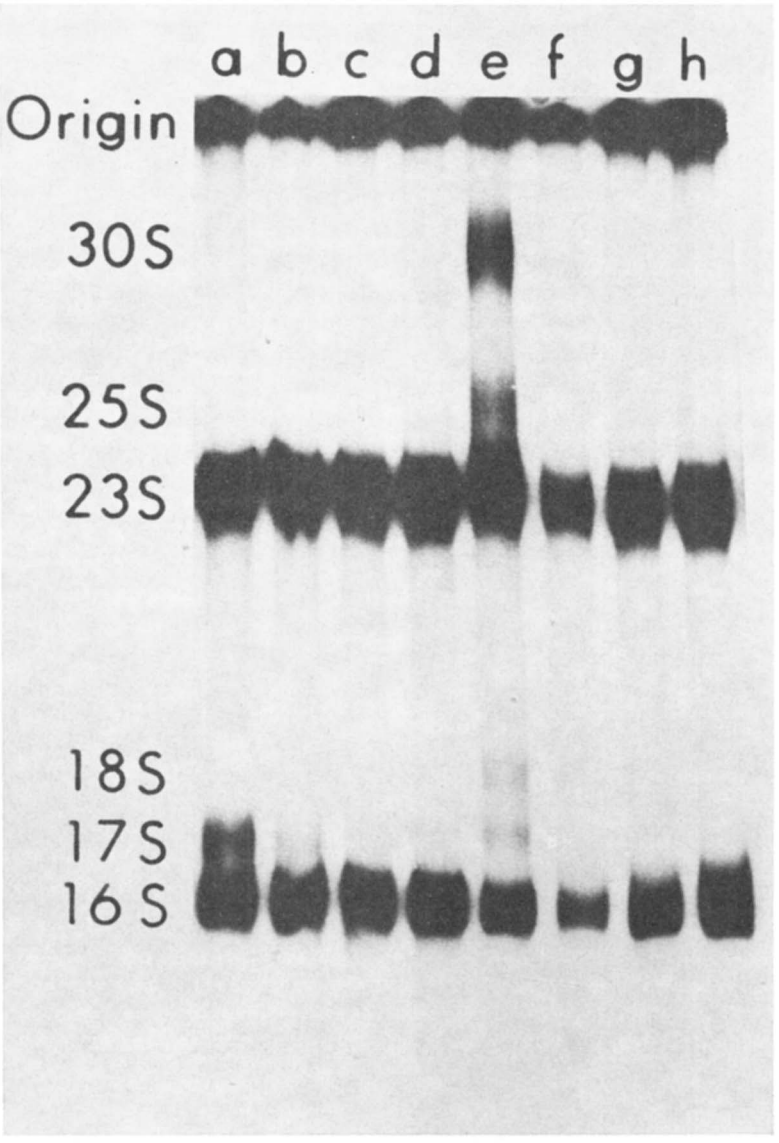

FIGURE 5.—Maturation of rRNA in an *rnc rho* mutant. Cells were grown in low phosphate rich medium (GEGENHEIMER, WATSON and APIRION 1977). Lanes a-d are from strain N2113 (*rnc+ rho-102*). Lane a contains material from cells labeled for 20 min with 50 μC/ml ^{32}Pi at 37°. For lanes b through d, a culture at A_{560} of 0.35 was transferred from 37° to 43° for 120 min and labeled with 100 μCi/ml of ^{32}Pi for 20, 40 and 60 min, respectively. Lanes e through h correspond exactly to lanes a through d except that strain N2336 (*rnc 105 rho-102*) was used. The cells were lysed and electrophoresed in a 3% polyacrylamide gel that was dried for autoradiography (APIRION, NEIL and WATSON 1976b; GEGENHEIMER, WATSON and APIRION 1977).

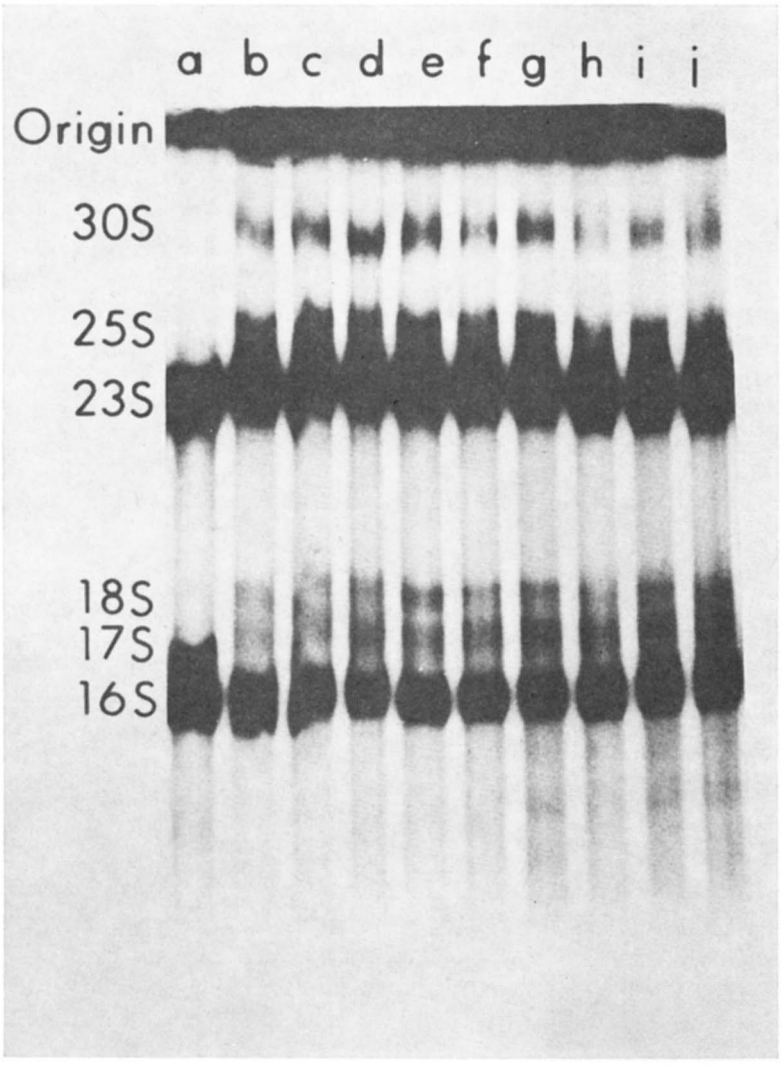

FIGURE 6.—Maturation of rRNA in various *rnc rho* mutants. Cells were grown in tris medium supplemented with 0.6% peptone, 0.2% glucose (GEGENHEIMER, WATSON and APIRION 1977), thiamine, nicotinic acid, and tryptophan. In each lane, material is displayed from cells labeled for 30 min with 50 μCi/ml of ^{32}Pi. Cells were lysed and processed as previously described (GEGENHEIMER, WATSON and APIRION 1977). Lane a contains material from strain N2112 (*rnc+ rho-101*) at 37°. For lanes b through d, three cultures of strain N2335 (*rnc-105 rho-101*) were grown to an A_{560} of 0.35 at 37°, transferred to 43°, and labeled after 90, 120 and 150 min respectively for 30 min. Lanes e, f, g and h, i, j contain material from strains N2336 (*rnc-105 rho-102*) and N2337 (*rnc-105 rho-103*) respectively, and are analogous to lanes b, c, and d. The cells were lysed and their content analyzed in a 3% polyacrylamide gel. The difference in the ratios of the different rRNA molecules in the experiments depicted in Figures 5 and 6 is related to the fact that cells grew faster in the medium used in Figure 5 (see GEGENHEIMER and APIRION 1975; GEGENHEIMER, WATSON and APIRION 1977).

and the level of decay of RNA was measured up to one hour after the label was added. (For details about such experiments, see PATO and VON-MEYENBURG 1970). All strains were tested at 30° for two hrs after they were shifted from 30° to 43°. Usually, as expected, 40 to 60% of the RNA incorporated was not degraded. This is the portion of the RNA that is rRNA and tRNA (PATO and VON-MEYENBURG 1970); however, in the case of *rnc rho* strains when RNA was labeled at 43°, about 80 to 90% of the RNA was degraded, indicating that rRNA is indeed being lost (*rnc*$^+$*rho*$^+$, *rnc rho*$^+$, *rnc*$^+$ *rho* and *rnc rho* strains were tested).

To explain these results, two obvious possibilities may be considered; (1) either there is an increased decay of rRNA in the doubly mutant strains, or (2) the newly synthesized rRNA fails to mature into ribosomes and is preferentially lost. The latter could result from an improper maturation of the rRNA, caused for instance by a lack in a particular ribosomal protein. The experiments described below suggest that in the double mutant strains at the elevated temperature there is an increased decay of rRNA. In order to distinguish between these two possibilities, cells of parental and doubly mutant strains were grown and labeled at 37° and 43° with [^{14}C] and [^{3}H]-uracil, respectively. Cells were mixed, gently lysed and layered on top of sucrose gradients (RON, KOHLER and DAVIS 1966). In such an experiment, if the rRNA synthesized at 43° enters into proper ribosomes, then the patterns of the two labels should coincide. On the other hand, if the newly synthesized rRNA does not enter ribosomes, then the patterns should not coincide, and the ^{3}H label should appear preferentially toward the top of the gradient. In numerous experiments including all the *rnc rho* strains that we have, the results showed clearly that the newly synthesized rRNA is processed and enters mature ribosomes, which most likely participate in protein synthesis. Sucrose-gradient analysis was carried out only up to 60 minutes after the shift of cultures to 43° in order to prevent the possibility of rRNA failing to mature into ribosomes because of a deficiency in protein synthesis (PACE 1973). In Figure 7, one such experiment is shown, in which cells of strain N2324 (*rnc rho*) were labeled at 37° for four hr with [^{14}C]-uracil, transferred to 43° for 60 min and labeled with [^{3}H]-uracil for 30 min. It can be seen that the RNA labeled at 43° cannot be distinguished from RNA labeled at 37°.

DISCUSSION

Such proteins as RNase III and rho affect the 3′ ends of RNA molecules (see Introduction); therefore it was of interest to find out if RNA metabolism is impaired in some way in the doubly mutant strains. If for instance these two proteins were interchangeable in their capacity to determine the 3′ ends of rRNA molecules, we would expect that in the double mutants the production of rRNA would be affected and longer rRNA molecules would be observed. If, on the other hand, rho cannot replace RNase III in the determination of the 3′ ends of rRNA molecules, we would expect that production of rRNA molecules would be very similar in *rnc rho*$^+$ and *rnc rho* strains. The results obtained in the experiments presented here suggest the latter, *i.e.*, rho does not substitute for RNase III

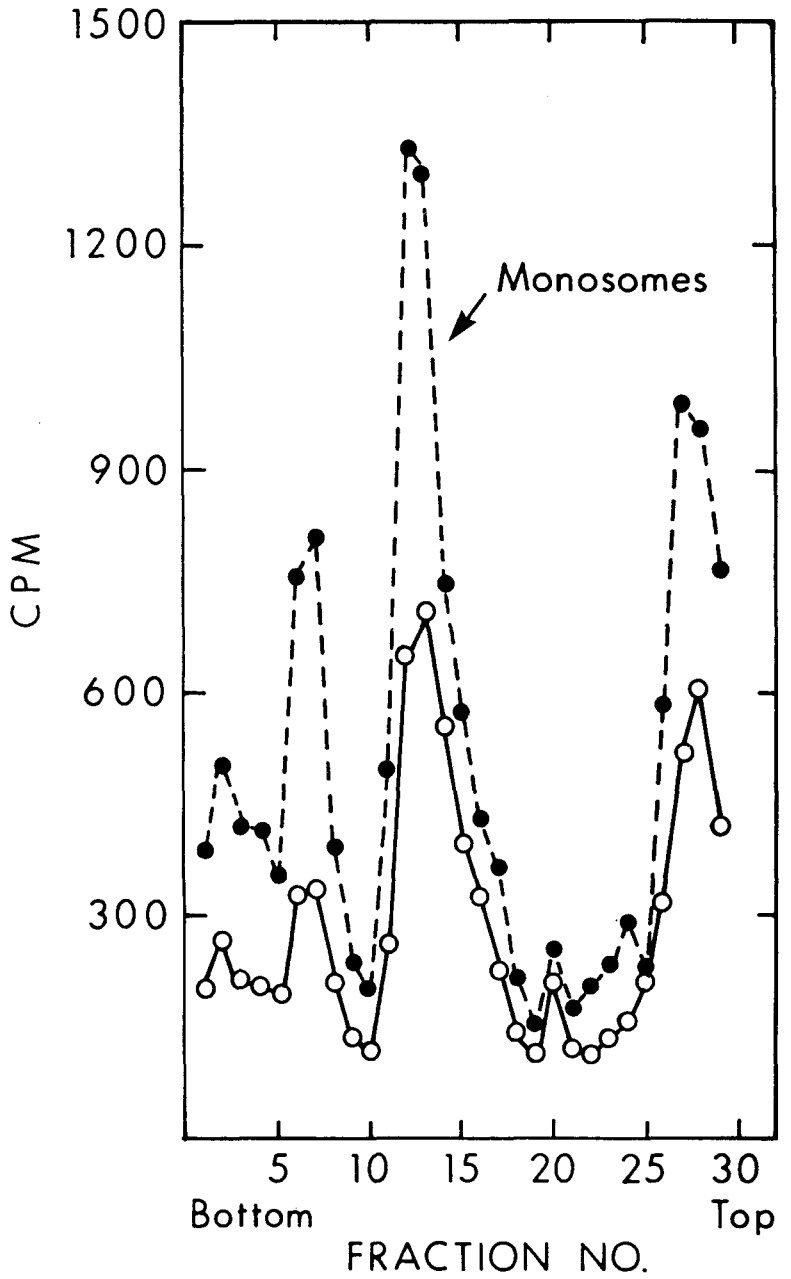

FIGURE 7.—Synthesis of ribosomes in an *rnc rho* strain at permissive and nonpermissive temperatures. A 30 ml culture of strain N2324 (*rnc-105 rho-101*) was grown at 37° in the same medium as in Figure 5, containing 3 μC of [2-^{14}C]-uracil (50 mC/mmole). After four hr while the cells were still in mid-log phase the culture was transferred to 43° and 60 minutes later 150 μC of [5,6-^{3}H]-uracil (45 C/mmole) were added. Thirty min later growth was stopped and content of cells was displayed on a 15–30% linear sucrose gradient containing 15 mM Mg $(CH_3COO)_2$. The experimental details were similar to those described by RON, KOHLER and DAVIS (1966) O—O; ^{14}C; ●—●, ^{3}H.

in the determination of the 3′ ends of rRNAs. Moreover, numerous scannings of gels from material labeled under different conditions failed to reveal any difference in the pattern of RNA synthesized in the doubly mutant strains as compared to the parental strains. Thus these experiments suggest that RNase III and rho do not substitute in the determination of the 3′ ends of any RNA molecule.

Our experiments demonstrate that the combination of *rnc rho* mutations in a single strain is viable. However, at elevated temperatures this combination becomes deleterious to the cell and growth stops. At the nonpermissive temperatures, message as well as rRNA and tRNA are synthesized, but the rRNA, even though it is being synthesized and processed and packaged into ribosomes, fails to accumulate and is degraded. *A priori*, it is hard to comprehend why stability of ribosomes should be affected by these two proteins, but a more thorough consideration of a number of observations can explain these findings rather satisfactorily.

The ribosome contains a large number of proteins (54), which among other functions also help to protect the rRNA from degradation by cellular ribonucleases. However, two thirds of the mass of the ribosome consists of RNA, and a good part of the rRNA is accessible to ribonucleases *in vitro*. *In vivo* the rRNA is stable only during exponential growth, while during various starvations the rRNA is suceptible to nuclear attack. The attack on the rRNA seems to be initiated by an endoribonuclease that exists in exponentially growing cells, and it seems to take place when the rRNA is in the ribosomal subunit that still contains all or most of the ribosomal proteins (KAPLAN and APIRION 1975a,b). Because of this, we expect that other nonribosomal proteins that can bind to rRNA have an effect on the stability of the rRNA. Indeed, we observed earlier (APIRION and WATSON 1974; APIRION, NEIL and WATSON 1976a) that in *rnc* cells the decay of rRNA during carbon starvation is accelerated. We explained this phenomenon by assuming that RNase III and the enzyme responsible for the initiation of degradation of rRNA (KAPLAN and APIRION 1975a,b), which could be RNase N (MISRA, RHEE and APIRION 1976), can bind to similar sites on the rRNA. Since RNase III cannot cut these sites, its binding to these rRNA sites provides some protection to the rRNA. In a similar way, it can be argued that the rho protein can attach to RNA (RICHARDSON 1970) and thus provide protection from ribonucleases. These considerations suggest that these two proteins, rho and RNase III, might provide protection to rRNA from degradation by virtue of their ability to bind to RNA molecules. Thus, these considerations suggest that the cause for the interaction between proteins RNase III and rho is rather nonspecific. We therefore suggest that in their action on determining the 3′ ends of RNA molecules, RNase III and rho do not complement each other, *i.e.*, rho cannot substitute for RNase III and *vice versa*. This suggestion is also supported by the findings that in *rnc rho*$^+$ strains the ends of all the rRNA precursor molecules seem to be abnormal since the RNase III cuts are not made (GEGENHEIMER, WATSON and APIRION 1977), which again suggests that *rho* cannot substitute for RNase III in determining the 3′ ends of rRNA precursor molecules. Therefore, we suggest that the mechanism of action of rho and RNase III is very different. Rho stops an RNA chain from growing and thus causes termination

(ROBERTS 1969), while RNase III can act only on an existing RNA chain. Our results also suggest that the nucleotide sequences recognized by these two proteins are not the same.

The genetic analyses indicate that the combination of the two mutations, *rnc* and *rho*, is necessary and sufficient for the interaction observed. This is implied by the fact that, in the transductions (see Table 2), all the *rho* transductants, and only those transductions, became Ts$^-$ when the recipient is *rnc*. Barring the unlikely possibility that all the donor strains tested (see Table 2) carry a mutation that is very closely linked to *rho* and that is necessary for this interaction, only the effect of the *rho* mutations was likely to be observed in the double mutants. Moreover, since one of the *rho* mutations, *rho-100*, which came from a very different background, behaved in the same way as *rho-101*, *102* and *103*, this possibility can be ignored. It is true that *rho-100 rnc-105* is less temperature sensitive than the other combinations, but this probably reflects the fact that it is a less extreme *rho* mutation when tested for relief of polarity in the *trp* or the *lac* operons (KORN and YANOFSKY 1976a). Since removal of the *rnc* mutations eliminated the temperature sensitivity, this suggests that other mutations, not closely linked to *rnc*, cannot be involved in the phenomena described here.

While these two mutations are necessary for the interaction, other mutations can probably also influence it. This can be assessed from the differences observed between strains N2324 and N2335. Both of these strains carry the *rnc-105* and the *rho-101* alleles, but since the first strain came from a mating experiment while the second came from a transduction involving different strains (see Table 1), not all of the genes are identical in these two strains; the differences between the two strains (as can be seen in Figure 4) can be attributed to the nonuniformity of their genetic background.

If both these proteins, rho and RNase III, have the capacity to bind to RNA molecules and thus provide them with some protection from cellular ribonucleases, why should double mutant strains *rnc rho* be viable at lower temperatures? The reasons for this seem to lie within the mechanism of rRNA decay and the nature of the *rho* mutations. While the nature of the *rnc-105* mutation is not known (missense, nonsense, etc.), a variety of experiments suggest that extracts of *rnc-105* strains do not contain any RNase III activity, and moreover the physiological effects of the *rnc-105* mutation are very similar at 30°, 37° or 43° (APIRION, NEIL and WATSON 1976a,b). The *rho* mutations studied here definitely contain rho protein (RICHARDSON, GRIMLEY and LOWERY 1975; KORN and YANOFSKY 1976b). However, the three *rho* mutants, *rho-101*, *-102* and *-103*, confer temperature sensitivity on their carrier strains at 45° (unpublished observations). The reason for the temperature sensitivity at 45° was not investigated. DAS, COURT and ADHYA (1976) isolated conditional lethal rho mutants and found a number of physiological defects in such strains. It is not clear which of these defects, if any, led to the lethality; degradation of rRNA was not tested. It is not obvious that strains defective only in rho would be affected in the stability of their rRNA; the various considerations presented here would suggest that

this is probably not the case. When our studies on *rnc rho* mutants were initiated, we were not aware of the conditional lethal rho mutants isolated by DAS, COURT and ADHYA (1976). Thus, the possibility exists that the mutant rho proteins show some thermolability in their protective function of RNA. It was noticed earlier that the decay of ribosomes is acceleraed at higher temperatures, 43° to 50°, as compared to 37° (KAPLAN and APIRION 1975a). Moreover, the enzyme RNase N, which is a good candidate for initiation of ribosome decay, degrades ribosomes faster at 43° than at 37° (MISRA, RHEE and APIRION 1976). Thus, even small differences in the amount of protection provided to ribosomes could be critical, and in the *rnc rho* double mutants this critical point is probably reached around 43°.

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LITERATURE CITED

APIRION, D., 1966 Altered ribosomes in a suppressor strain of *Escherichia coli*. J. Mol. Biol. **16**: 285–301.

APIRION, D. and N. WATSON, 1974 Analysis of an *Escherichia coli* strain carrying physiologically compensating mutations one of which causes an altered ribonuclease III. Molec. Gen. Genet. **132**: 89–104. ——, 1975 Mapping and characterization of a mutation in *Escherichia coli* that reduces the level of ribonuclease III specific for double-stranded ribonucleic acid. J. Bacteriol. **124**: 317–324.

APIRION, D., J. NEIL and N. WATSON, 1976a Consequences of losing ribonuclease III on the *Escherichia coli* cell. Molec. Gen. Genet. **144**: 185–190. ——, 1976b Revertants from RNase III negative strains of *Escherichia coli*. Molec. Gen. Genet. **149**: 201–210.

BACHMAN, B., B. LOW and A. L. TAYLOR, 1976 Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**: 116–167.

DAS, A., D. COURT and S. ADHYA, 1976 Isolation and characterization of conditional lethal mutants of *Escherichia coli* in transcription termination factor *rho*. Proc. Natl. Acad. Sci. U.S. **73**: 1959–1963.

DE CROMBRUGGHE, B., S. ADHYA, M. GOTHESSMAN and I. PASTAN, 1973 Effect of Rho on transcription of bacterial operons. Nature New Biol. **241**: 260–264.

DUNN, J. J. and F. W. STUDIER, 1973a T7 early RNAs are generated by site-specific cleavages. Proc. Natl. Acad. Sci. U.S. **70**: 1559–1563. ——, 1973b T7 early RNAs and *Escherichia coli* ribosomal RNAs are cut from large precursor RNAs *in vivo* by ribonuclease III. Proc. Natl. Acad. Sci. U.S. **70**: 3296–3300.

GEGENHEIMER, P. and D. APIRION, 1975 *Escherichia coli* ribosomal ribonucleic acids are not cut from an intact precursor molecule. J. Biol. Chem. **250**: 2407–2409.

GEGENHEIMER, P., N. WATSON and D. APIRION, 1977 Multiple pathways for the primary processing of ribosomal RNA in *Escherichia coli*. J. Biol. Chem. **252**: 3064–3073.

GINSBURG, D. and J. A. STEITZ, 1975 The 30S ribosomal precursor RNA from *Escherichia coli*. J. Biol. Chem. **250**: 5647–5654.

KAPLAN, R. and D. APIRION, 1975a The fate of ribosomes in *Escherichia coli* cells starved for a carbon source. J. Biol. Chem. **250**: 1854–1863. ——, 1975b Decay of ribosomal ribonucleic acid in *Escherichia coli* cells starved for various nutrients. J. Biol. Chem. **250**: 3174–3178.

KORN, L. J. and C. YANOFSKY, 1976a Polarity suppressors increase expression of the wild-type tryptophan operon of *Escherichia coli*. J. Mol. Biol. **103**: 395–409. ——, 1976b Polarity suppressors defective in transcription termination at the attenuator of the tryptophan operon of *Escherichia coli* have altered *rho* factor. J. Mol. Biol. **106**: 231–241.

LENNETTE, E. T. and D. APIRION, 1971 Genetic analysis of an *Escherichia coli* syndrome. J. Bacteriol. **108**: 1322–1328.

LOZERON, H. A., P. J. ANEVSKI and D. APIRION, 1977 Antitermination and absence of processing of the leftward transcript of coliphage lambda in the RNase III deficient host. J. Mol. Biol. **109**: 359–365.

MILLER, J. H., 1972 *Experiments in molecular genetics*. Pub. Cold Spring Harbor Laboratory.

MISRA, T. K., S. RHEE and D. APIRION, 1976 A new endoribonuclease from *Escherichia coli*. J. Biol. Chem. **251**: 7669–7674.

MORSE, D. E. and M. GUERTIN, 1972 Amber SuA mutations which relieve polarity. J. Mol. Biol. **63**: 605–608.

PACE, N. R., 1973 Structure and synthesis of the ribosomal ribonucleic acid of prokaryotes. Bacteriol. Rev. **37**: 562–603.

PATO, M. L. and K. VON MEYENBURG, 1970 Residual RNA synthesis in *Escherichia coli* after inhibition of initiation of transcription by rifampicin. Cold Spring Harbor Symp. Quant. Biol. **35**: 497–504.

RICHARDSON, J. P., 1970 *Rho* factor function in T4 transcription. Cold Spring Harbor Symp. Quant. Biol. **35**: 127–133.

RICHARDSON, J. P., C. GRIMLEY and C. LOWERY, 1975 Transcription termination factor *Rho* activity is altered in *Escherichia coli* with SuA gene mutations. Proc. Nat. Acad. Sci. U.S. **72**: 1725–1728.

ROBERTS, J., 1969 Termination factor for RNA synthesis. Nature **224**: 1168–1174.

RON, E. A., R. E. KOHLER and B. D. DAVIS, 1966 Polysomes extracted from *Escherichia coli* by freeze-thaw-lysozyme lysis. Science **153**: 1119–1120.

STUDIER, F. W., 1975 Genetic mapping of a mutation that causes ribonuclease III deficiency in *Escherichia coli*. J. Bacteriol. **124**: 307–316.

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